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Investigation of the Translational Frameshift within EA Bacteriophage Tail Assembly Chaperones

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Translational frameshifting is a mechanism by which an organism can produce a short and long form of a specific protein and is highly conserved within the tail assembly chaperone (TAC) genes of dsDNA tailed bacteriophages. Bacteriophages modulate the frequency of frameshifting to achieve the desired ratio of the two protein isoforms and in most cases, the frameshifted protein is only produced ~4% of the time.   
  
The University of Ottawa’s Phage Hunters course has found four EA cluster phages that infect *Microbacterium foliorum*: Winzigespinne (EA1), Erla (EA1), Strathdee (EA1) and Quartz (EA10). These phages as well as phages from the EA subclusters EA2, EA7, EA8, and EA9, do not annotate a potential programmed translational frameshift within the TAC genes. This is due to insufficient experimental evidence to identify and confirm the “slippery sequence” that would cause the frameshift within these phages.   
  
Here we present evidence that bacteriophage Winzigespinne undergoes a translational frameshift between gene 15 and 16 within the TAC genes. We have raised polyclonal antibodies to fragments of gene 16 that specifically detect the frameshifted TAC protein and used these antibodies to prove that the frameshift occurs in vivo during bacteriophage infection. In addition, we have used a heterologous expression system in E. coli to show that GST-tagged TAC genes from several EA phages produce short, long and read-through TAC proteins. We have begun to use this system to define the position of the “slippery sequence” via mutagenesis of four proposed “slippery sequence” sites. Due to the conservation of the TAC among many phage clusters, this work can be used to examine previously annotated and proposed frameshifts, and to determine if there are cluster-specific differences in the extent of frameshifting during infection.